THE INFLUENCE OF ABIOTIC AND BIOTIC ENVIRONMENTAL FACTORS AT HIGH ELEVATION ON THE LIFE CYCLE OF A FACULTATIVELY PAEDOMORPHIC SALAMANDER

A thesis presented to the faculty of the Graduate School of Western Carolina University in partial fulfillment of the requirements for the degree of Master of Science in Biology

By

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ABSTRACT

THE INFLUENCE OF ENVIRONMENTAL FACTORS AT HIGH ELEVATION ON THE LIFE CYCLE OF A FACULTATIVELY PAEDOMORPHIC SALAMANDER

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Environmentally cued polymorphisms are excellent models for studying the origins of phenotypic plasticity. Facultative paedomorphosis in salamanders is a form of discrete phenotypic plasticity in which aquatic larvae respond to environmental cues by either metamorphosing into a terrestrial subadult (complex life cycle) or maturing as a paedomorph in the aquatic larval body form (simple life cycle). There are two proposed selective mechanisms for the maintenance of paedomorphosis in salamanders: (1) "paedomorph advantage" (PA), in which a favorable aquatic habitat offers greater fitness to larger individuals than the terrestrial habitat and smaller individuals metamorphose to escape competition and (2) "best of a bad lot" (BBL), in which small individuals maximize fitness in a growth-limiting environment by forgoing metamorphosis thereby allowing reproduction during the first breeding season and large individuals metamorphose to escape the suboptimal aquatic habitat. In this study, I examined the influence of abiotic and biotic factors on the life cycles of a facultatively paedomorphic salamander, *Ambystoma talpoideum*, at the highest elevation within its range in the southern Appalachian Mountains in western North Carolina. This species has been studied extensively at low elevations, but the selective factors of the environment may be different at high elevations. This study consisted of three parts: (1) field surveys to characterize the breeding phenology and population structure, (2) a mesocosm experiment manipulating temperature to determine the selective mechanism of facultative paedomorphosis in these populations, and (3) aggression trials between metamorphic *A. talpoideum* and a fully terrestrial

salamander endemic to this region to explore the potential for competitive exclusion of metamorphic *A. talpoideum* in the terrestrial environment. Field surveys revealed that these high-elevation populations are majority paedomorphic, and paedomorphs can breed after metamorphs have left the wetland. While temperature did not have an effect on the final body size of larvae in the mesocosm experiment, density did. No individuals metamorphosed before the first breeding season, but some metamorphosed the following spring. Individuals that grew to a larger body size were more likely to metamorphose, which supports the BBL selective mechanism for facultative paedomorphosis in these populations. Aggression trials found low levels of aggression between metamorphic *A. talpoideum* and the fully terrestrial salamander, *Plethodon shermani*. This might be because these two species inhabit different spatial niches as they use burrows at different depths. This study suggests that facultative paedomorphosis can have different adaptive mechanisms under a different regime of environmental conditions.

CHAPTER I: LIFE CYCLES OF A FACULTATIVELY PAEDOMORPHIC SALAMANDER AT HIGH ELEVATION

INTRODUCTION

Facultative paedomorphosis in salamanders is a polyphenism that features two alternative life-history pathways: a complex life cycle (CLC) and a simple life cycle (SLC). CLCs involve a rapid transformation between successive body forms distinct in morphology, physiology, and behavior (Wilbur, 1980). They are found in a variety of animal taxa including insects (Sano and Ozaki, 2012; Stoks and Cordoba-Aguilar, 2012), crustaceans (Twombly, 1996), and amphibians (Wilbur, 1980; 1984). The transformation, or metamorphosis, exhibited in CLCs typically includes a shift in habitat and ecological niche (Werner, 1986; Nielsen, 1998). In amphibians, this usually consists of an aquatic larva metamorphosing into a terrestrial subadult. This ontogenetic niche shift allows larvae to capitalize on ephemeral habitats and resources, and it also allows both stages to specialize in the two different environments (Wilbur and Collins, 1973). This habitat partitioning reduces resource competition between the metamorphic and larval forms (Takimoto, 2003). However, metamorphosis is energetically costly and can be risky due to uncertainty about the quality of the subsequent habitat (Wilbur and Collins, 1973). Therefore, phenotypic plasticity in metamorphosis is expected to be common in species with CLCs, because it allows individuals to respond to environmental variability in a way that maximizes fitness (Denoël et al., 2005). In amphibians, CLCs are thought to be a conserved trait (Moran, 1994), while SLCs are derived (Ryan and Semlitsch, 2003; Mueller et al., 2004).

In salamanders, paedomorphosis is a type of SLC that is an alternative life-history pathway to a CLC. Paedomorphosis is the retention of the larval aquatic morphology (gills, tail fin, and other traits) while attaining reproductive maturity (Gould, 1997). This trait can arise via either arrested somatic development relative to reproductive development (neoteny) or an increase in reproductive development relative to the rest of the body (progenesis; Denoël and Joly, 2000). In some species paedomorphosis is

obligate, but in others it is facultative. In facultative paedomorphosis, the life-history pathway a larva follows is dependent on an interaction between its genes and the environmental conditions it experiences (Duellman and Trueb, 1986). Thus, larvae may respond to environmental cues by either metamorphosing or maturing as a paedomorph. This polyphenism allows individuals the opportunity to follow the life-cycle pathway that will lead to maximal fitness under the current environmental conditions.

As facultative paedomorphosis elucidates the effects of the environment on the balance in fitness tradeoffs between CLCs and SLCs, it has been the subject of much theoretical and empirical study. Wilbur and Collins (1973) first conceived a mechanism describing how environmental factors can select for and maintain facultative paedomorphosis in salamanders. Whiteman (1994) expanded upon this mechanism and described an additional mechanism. Both are based on the relative quality of the aquatic and terrestrial environments and the expected fitness that individuals of different body sizes can expect under a given environmental regime. Under the "paedomorph advantage" (PA) mechanism, larger individuals will remain as paedomorphs in an aquatic environment that is favorable (e.g. low conspecific density, high food availability, optimal temperature, and low predation pressure) in order to continue capitalizing on the quality habitat, while slower growing individuals will maximize their fitness by metamorphosing to escape interspecific competition with larger larvae and paedomorphs. "Best of a bad lot" (BBL) is an alternative mechanism, in which larger individuals metamorphose to escape poor growing conditions in the aquatic habitat (e.g. high conspecific density, low food availability, suboptimal temperature, and high predation pressure). Smaller individuals that are not able to reach the minimum size for metamorphosis before their first breeding season are able to increase their fitness by investing their limited energy in reproduction as a paedomorph in order to breed earlier. Most experimental evidence supports the PA mechanism, probably because a majority of these studies have been carried out in favorable aquatic conditions (Denoël et al., 2005). Support for PA has been found in individuals from low-elevation *Ambystoma talpoideum* populations in South Carolina (Semlitsch, 1987a,b; Jackson and Semlitsch, 1993; Ryan and Semlitsch, 1998). The few occurrences of evidence supporting BBL have been in locations where the temperatures are lower and the growing season is shorter because the populations

are either at high elevations (*Ambystoma gracile*; Sprules, 1974 and *A. tigrinum*; Whiteman et al., 1996) or the northern extent of the species' range (*Ambystoma talpoideum*; Doyle and Whiteman, 2008).

The full potential of *Ambystoma talpoideum* as a model organism for studying the influence of the environment on the relative fitness payoffs involved in facultative paedomorphosis has yet to be realized. This species has been studied extensively at low elevations in South Carolina (Semlitsch, 1985a,b, 1987a,b; Jackson and Semlitsch, 1993; Ryan and Semlitsch, 1998, etc.) and the northern extent of its range in Kentucky (Doyle and Whiteman, 1974), but not at the highest elevation in its range. This study seeks to expand our understanding of the evolution and maintenance of facultative paedomorphosis by studying *A. talpoideum* at the highest elevational extent of its range in the southern Appalachian Mountains in western North Carolina. The selective factors at these sites may be different than at the low-elevation sites where this species has been previously studied. At high elevation sites the annual mean temperature is lower, precipitation is higher, and the wetlands are typically permanent (Table 1). Lower temperature at high elevations likely limits growth in the aquatic environment. Thus, the aquatic environment may be less favorable for *A. talpoideum* at high elevations than at low elevations. Higher annual precipitation in the southern Appalachian Mountains, along with lower sand content in the soil probably increases soil moisture, which would make the terrestrial environment more favorable at high elevations than low elevations in the South Carolina coastal plain. Therefore, the habitat quality of the terrestrial environment, relative to the aquatic environment, could be more favorable for *A. talpoideum* at the high elevations in the mountains of North Carolina, while the opposite could be true at low elevations in South Carolina.

This study had two objectives: (1) to characterize the breeding phenology and relative proportion of the two adult morphologies of *A. talpoideum* at high elevations using field surveys and (2) to determine the selection mechanism that maintains facultative paedomorphosis in these populations using a mesocosm experiment that artificially manipulated temperature. The aim of the first objective was to increase knowledge about the natural history of the high-elevation populations, so I did not make any explicit predictions about the field surveys. In the mesocosm experiment, I predicted that individuals in

the control treatment that were larger would metamorphose and smaller individuals would become paedomorphs under the BBL mechanism, because of the environmental factors associated with high elevations that could limit larval growth (e.g. suboptimal temperature, constricted growing season, etc.). In the warming treatment, I expected either: (1) increasing temperature would increase growth rates, which would allow more larvae to grow larger and metamorphose under the BBL mechanism, while smaller larvae would still remain paedomorphic or (2) the increased temperature would create a more favorable habitat for growth, which would result in larger larvae becoming paedomorphic and smaller individuals metamorphosing under the PA mechanism.

Table 1. Environmental factors at study sites in Macon County, North Carolina compared to a location of previous studies on *Ambystoma talpoideum* at low elevations in Aiken and Barnwell Counties, South Carolina.

Environmental factor	**High elevations[1,2] (Macon Co., NC)**	**Low elevations[3] (Aiken and Barnwell Cos., SC)**
Elevation range	945 – 1035 m	75 – 200 m
Annual temperature mean	12.9 ºC	17.3 ºC
Annual rainfall mean	180 – 238 cm	119 cm
Pond hydroperiod	permanent[4]	variable[5]

[1]Temperature data at Coweeta LTER (670 m elevation) 1935 – 2015 (Miniat et al., 2015)
[2]Rainfall data at Coweeta LTER (670 and 1340 m elevation) 1935 – 2015 (Miniat et al., 2015)
[3]Annual climate normals for Barnwell, South Carolina 1981 – 2010 (Arguez et al., 2010)
[4]Pers. obs.
[5]Semlitsch and Gibbons, 1985

MATERIALS AND METHODS

Study Sites

The high-elevation *Ambystoma talpoideum* populations examined in this study occur in permanent wetlands along the headwaters of the Nantahala River in the Nantahala Mountains of Macon County, North Carolina. The activities in this study occurred at two wetlands: Whiteoak Bottoms, a large wetland complex mediated by beaver activity in the Nantahala National Forest (1035 m elevation); and Thunderstruck Wetland, a smaller spring-fed wetland on private property (945 m elevation). Whiteoak Bottoms contains redbreast sunfish (*Lepomis auritus*) and both wetlands contain creek chubs (*Semotilus atromaculatus*).

Field Surveys

Terrestrial surveys.--- I surveyed the terrestrial habitat to characterize the phenology of metamorphic *A. talpoideum* breeding migrations. A drift fence (silt fencing, 70 m long, 1 m tall; Gibbons and Semlitsch, 1981) was installed along the northeast edge of the Whiteoak Bottoms wetland complex. Pitfall traps (14 cm deep, 12 cm diameter) equipped with small-mammal escape ropes were placed on both sides of the fence approximately every ten meters and were only open during surveys. Surveys were conducted opportunistically on rainy nights above 10 ºC (50 ºF) when *A. talpoideum* movement is greatest (Semlitsch, 1985b). I recorded the direction of travel (into or out of wetland), sex (if mature), and presence or absence of gill nubs for each individual encountered.

Aquatic surveys.--- I surveyed the aquatic habitat to characterize the population structure of high-elevation *A. talpoideum* populations. Dipnet surveys alternated monthly between Whiteoak Bottoms and Thunderstruck wetland from 27 May 2014 to 6 April 2015. These surveys were standardized by the number of one-meter long net sweeps within a constrained area (1 sweep/2 m^2). Net sweeps were in leaf litter that was at a depth of 0.25 – 1 m below the water surface. The survey area at Whiteoak Bottoms was 82 m^2 (41 net sweeps/survey), and at Thunderstruck wetland it was 130 m^2 (65 net sweeps/survey).

Preliminary surveys were also carried out that were not standardized. Each individual's snout-vent length (SVL), total length, mass, morphotype, and sex (as determined by cloacal swelling) was recorded.

Mesocosm Experiment

Experimental design.--- This experiment was designed to determine the effect of temperature on growth and the expression of the two adult morphotypes of *A. talpoideum*. Sixteen mesocosm tanks (plastic wading pools, 1.5 m diameter, 0.3 m deep, 0.5 m^3 volume) were arranged in two lines in four spatial blocks in a field on the campus of Western Carolina University in Cullowhee, North Carolina. From 27 – 30 April 2014, I filled the mesocosm tanks with an equal amount of tap water and leaves (400 g each) collected from upland habitat composed mostly of deciduous trees (*Liriodendron tulipifera*, *Quercus spp.*, *Fagus grandifolia*, and limited *Pinus strobus*). Mesh fiberglass screens were placed over the tanks to prevent oviposition by predators, such as odonates. After the first month, the screens were removed only at night to allow colonization by dipterans and other potential prey. During the summer, the screens were left on to prevent oviposition by tree frogs (mainly *Hyla chrysoscelis*). Aquarium heaters (ViaAqua 200 watt Quartz Heater; Commodity Axis Inc. Camarillo, California) were randomly assigned to half of the tanks and turned on 13 May 2014 to simulate environmental temperatures experienced by *A. talpoideum* populations at low elevations. To account for the structure the aquarium heaters provide for algal growth, test tubes of similar shape and size (surface area ~80%) were placed in the control tanks. I suspended a waterproof temperature logger (iBCod; Thermodata Corporation; Whitewater, Wisconsin) 10 – 20 cm from the bottom of each tank, and set them to record temperature hourly. Plankton was collected with a fine mesh net from natural wetlands, and 600 mL aliquots of the resulting concentrated plankton were randomly assigned to each tank on 5 May, 16 May, 27 May, 3 June, 14 July, and 16 August 2014.

Ambystoma talpoideum eggs were collected from Thunderstruck wetland near Rainbow Springs, North Carolina on four occasions from 6 – 26 April 2014 and kept outside in the shade where they were exposed to natural diel temperature fluctuations until hatching. I randomly assigned and gradually acclimated five larvae to each tank 30 May 2014. These larval densities (5 larvae/500 L = 10 larvae/1000L) were scaled to fit within the low density (6 larvae/1000L) and medium density (18

larvae/1000L) levels in a previous study (Semlitsch, 1987a). Once larvae were large enough (>17 mm SVL; Doyle and Whiteman, 2008; White, pers. obs.) I gave each a unique mark 27 – 29 July 2014 using visible implant elastomer tags (Northwest Marine Technologies; Tumwater, Washington). On nine occasions from 27 July 2014 to 6 May 2015, I collected salamanders with dipnets and measured each individual's SVL and total length to the nearest millimeter and mass to the nearest hundredth gram (Ohaus Scout Pro SP402; Ohaus Corporation; Pine Brook, New Jersey). I also noted sexual maturity and sex as determined by swelling of the cloaca (Whiteman and Semlitsch, 2005).

On 17 November 2014, I placed four plastic bags (~50 L each) filled with leaves around the outer edge of each tank for insulation. When air temperatures dropped below -7 ºC for extended time periods I covered the tanks with 0.31mil clear plastic to prevent the tanks from freezing completely. Surface ice was broken throughout the winter to prevent anoxic conditions. Metamorphic individuals were removed from the tanks and placed in containers with moist unbleached paper towels until they were released at the edge of the Thunderstruck wetland. The experiment concluded on 15 June 2016. Surviving paedomorphic and larval individuals were returned to the Thunderstruck wetland. Salamanders were not sacrificed because of their status as a species of special concern in North Carolina.

Statistical analyses.--- Analyses of early mesocosm experiments on facultative paedomorphosis generally used tank as the experimental unit (Semlitsch, 1987a, b; Jackson and Semlitsch, 1993; Ryan and Semlitsch, 1997). However, Doyle and Whiteman (2008) recognized the importance of examining this question at the individual level. I used generalized mixed-effects logistic regression for categorical response variables and generalized linear mixed-effects models for continuous response variables with tank and block included as random effects. Including tank in the models as a random effect allowed analysis of individual data (instead of tank means) while accounting for the issue of pseudoreplication (Millar and Anderson, 2004; Davies and Gray, 2015). I produced models to test the effect of the temperature treatment on survival, the effects of temperature and density (resulting from unequal early mortality among tanks) on SVL prior to the spring metamorphosis event, the effect of temperature and density on probability of metamorphosis, and the effect of SVL prior to the metamorphosis event on the

probability of metamorphosis. I used SVL during the last census event (March 2015) prior to the spring metamorphosis event that began May 2015 instead of SVL after the metamorphosis event in order to avoid the confounding effect of metamorphosis on growth. Analyses examining the probability of metamorphosis only included individuals that either metamorphosed or matured as paedomorphs. All analyses were performed in R (Version 3.1.3, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Field Surveys

Terrestrial surveys.--- I surveyed the drift fence opportunistically 12 times from 6 October 2014 to 9 June 2015. A total of 15 metamorphic *A. talpoideum* were observed migrating into the wetland on six occasions from 6 October to 23 December 2014, and 44 were observed moving out of the wetland on four occasions from 4 March to 9 June 2015 (Fig. 1). A majority of the metamorphs moving into the wetland were males (93%). As individuals left the wetland, many individuals no longer had swollen cloacas typical during the breeding season, which sometimes made determination of sex difficult or impossible. Of the individuals leaving the wetland that could be sexed, 61% were males. The individual observed on 9 June 2015 showed signs of recent metamorphosis (gill nubs), and its size (59 mm SVL) indicates that it was paedomorphic prior to metamorphosis. None of the other metamorphs leaving the wetland had gill nubs.

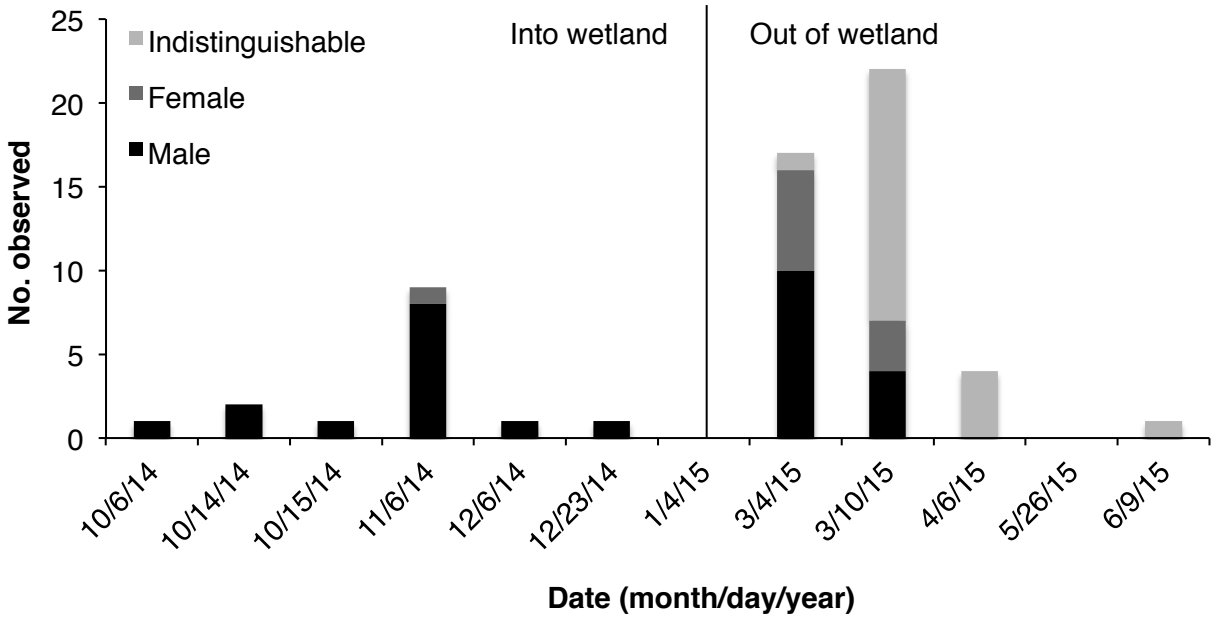

Fig. 1. Number of metamorphic *Ambystoma talpoideum* migrating into the wetland (6 October – 23 December 2014) and out of the wetland (4 March – 9 June 2015).

Aquatic surveys.--- Preliminary dipnet surveys from 17 November 2013 to 26 April 2014 were not standardized. Standardized dipnet surveys occurred 27 May 2014 to 6 April 2015 (Table A1). A total of 84 salamanders were recorded in dipnet surveys during the breeding season (October – April). A majority of individuals found were paedomorphic or larval, and only three metamorphic individuals (6% of adults) were found in the wetlands (Fig. 2). Paedomorphic females depositing eggs singly were observed 9 April 2014.

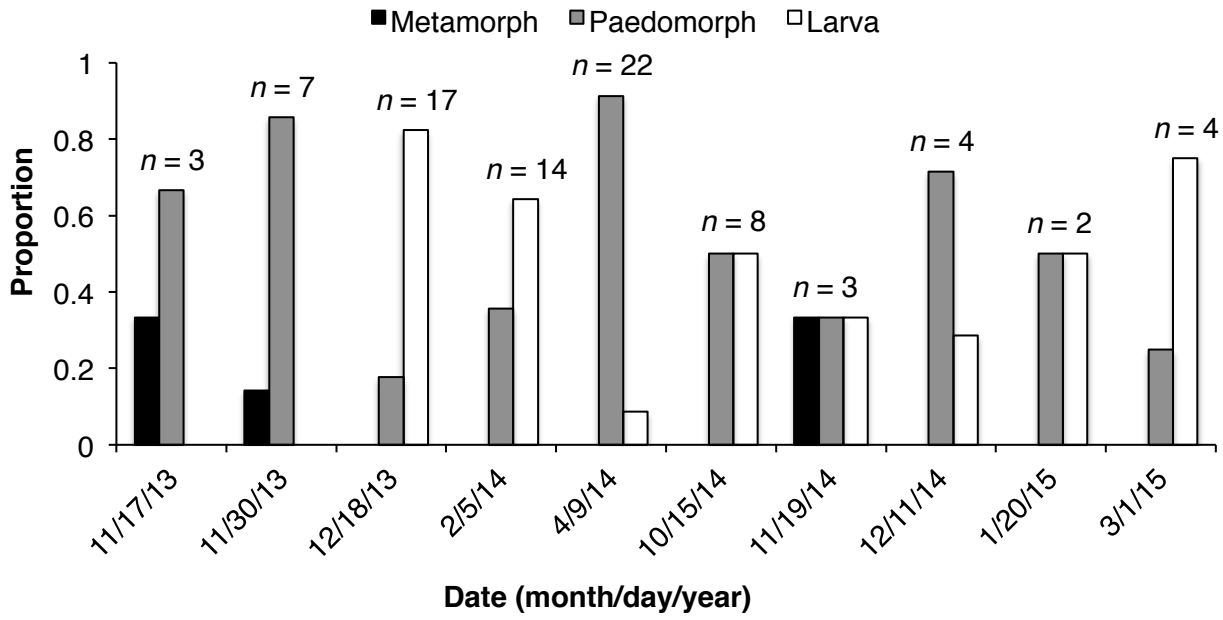

Fig. 2. Proportion of metamorphic, paedomorphic, and larval salamanders observed in the wetland over the course of two breeding seasons (October – April).

Mesocosm Experiment

Mesocosm tanks in the control treatment averaged 20.8 ºC in comparison to 22.7 ºC for the heated treatments (Appendix, Fig. A1). Mortality within the first two months of the experiment caused unequal densities among tanks (range = 2 – 5 individuals; mean = 3.4). Temperature did not show an effect on survival ($P = 0.47$). No salamanders metamorphosed before the first breeding season, though 31 of the surviving 54 (57%) matured sexually as paedomorphs. Eight individuals (15%) metamorphosed the following spring (May – June 2015), and six of these individuals were previously mature as paedomorphs. Temperature did not affect SVL prior to metamorphosis ($P = 0.32$), but density did ($P = 0.003$). Tukey's post hoc test revealed that individuals in tanks that contained two individuals had larger SVL than those in tanks of any other density (2 vs. 3, $P = 0.011$; 2 vs. 4, $P = 0.033$; 2 vs. 5, $P = 0.002$; Fig. 3). Metamorphosis probability was not significantly affected by temperature ($P = 0.71$) or density ($P = 0.097$). The average SVL of metamorphs was larger than individuals that remained paedomorphic or

larval (Fig. 4). When comparing metamorphs and paedomorphs, individuals that attained a larger SVL during the final census (March 2015) prior to the spring metamorphosis event had a higher probability of metamorphosing ($P = 0.035$; Fig. 5). Analyses replacing SVL with mass yielded qualitatively similar results for all models.

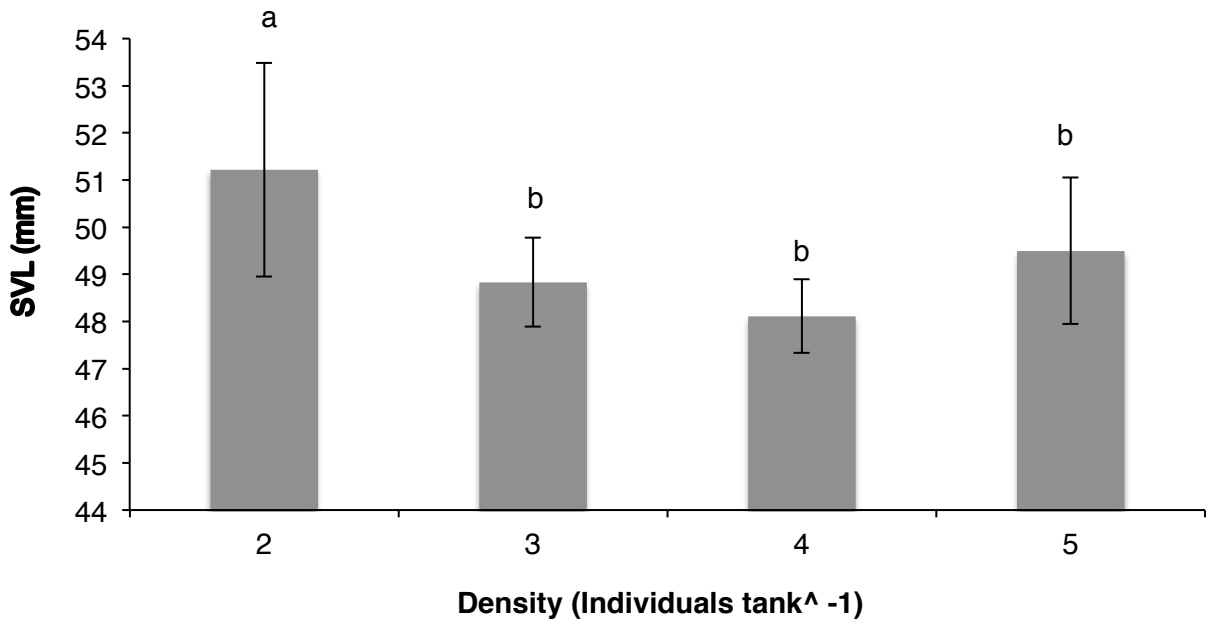

Fig. 3. Average final snout-vent length (SVL) ± SE of salamanders in tanks with various densities: 2, 3, 4, or 5 total individuals. Different letters represent Tukey's Post hoc $P < 0.05$.

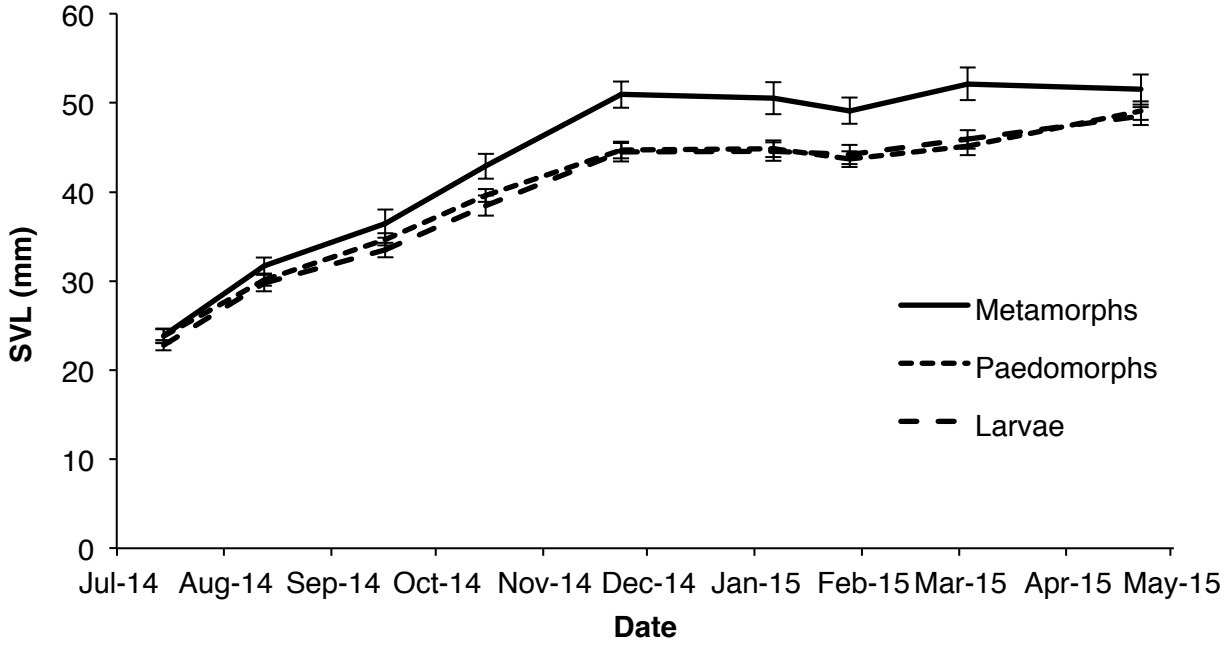

Fig. 4. Average snout-vent length (SVL) of *Ambystoma talpoideum* that metamorphosed and those that remained paedomorphic or larval at the end of the experiment. Error bars are ± SE.

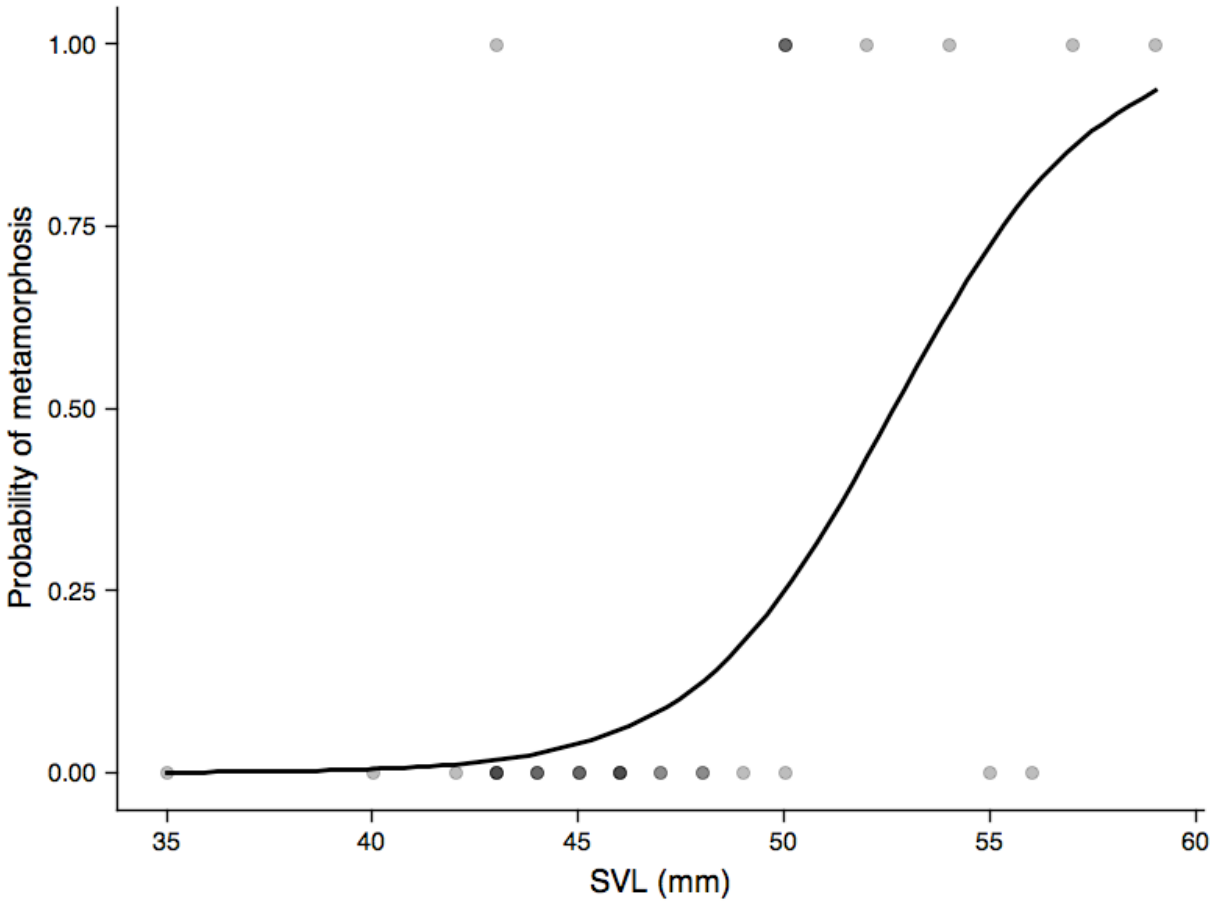

Fig. 5. Predictive logistic regression model of the probability of metamorphosis based on snout-vent length (SVL) prior to the spring metamorphosis event. Points represent raw data, and darker points indicate multiple observations. The SVL measurements of individuals that metamorphosed were 59, 57, 54, 52, 50, 50, 50, and 43, and the measurements of those that remained paedomorphic were 56, 55, 50, 49, 48, 48, 47, 47, 46, 46, 46, 46, 45, 45, 45, 44, 44, 44, 43, 43, 43, 43, 42, 40, and 35.

DISCUSSION

Terrestrial drift fence surveys showed that breeding migrations of metamorphic *A. talpoideum* at high elevations in the southern Appalachian Mountains of North Carolina occur during the same overall time frame (October – March) as at low elevations (90 – 200m) in Aiken and Barnwell Counties, South Carolina. However, there appear to be some differences within that time frame. In South Carolina, *A. talpoideum* migrations occur throughout the period from October to March (Whiteman and Semlitsch, 2005). Within this range, adequate weather conditions (rainfall and air temperatures above 10 ºC) are thought to have a greater influence on the timing of breeding migrations than calendar date (Semlitsch, 1985b). I speculate that breeding migrations in high-elevation populations may be less likely to occur during the coldest months of winter (usually January and February) when nighttime air temperatures above 10 ºC are rare. In the coastal plain, metamorphic females are fewer and arrive later than metamorphic males (Whiteman and Semlitsch, 2005). My observations of the male-biased sex ratio and earlier arrival of males in high-elevation populations are consistent with both of these findings (Fig. 1). Only one metamorph leaving the wetland had gill nubs, and its size indicated that it was likely paedomorphic prior to metamorphosis. All of the other metamorphs leaving the wetland must have either been metamorphic at the beginning of the breeding season or were paedomorphs that absorbed their gills completely before leaving the wetland.

The aquatic dipnetting surveys revealed that the high-elevation populations are majority paedomorphs. In the Atlantic Coastal Plain, populations in permanent ponds have a higher propensity to become paedomorphic than populations in ephemeral ponds, but the selective mechanism driving the evolution of this trait is unclear (Semlitsch and Gibbons, 1985; Semlitsch et al., 1990). Based on mathematical models proposed by Wakano and Whiteman (2008), the proportion of paedomorphs is expected to be high in two situations: (1) in a population maintained by BBL when paedomorph density is high and (2) in a population maintained by PA when paedomorph density is low. In a population maintained by PA, paedomorphosis becomes less beneficial (from a fitness standpoint) as the density of

paedomorphs increases because of increased competition for resources. In a population maintained by BBL, the increased competition caused by an increase in paedomorph proportion forces a high proportion of individuals into paedomorphosis. My study did not measure density, so I cannot infer the mechanism maintaining facultative paedomorphosis in these populations based solely on the relative proportion of the two morphs.

In coastal plain populations, paedomorphs have been observed breeding prior to arrival of metamorphs (Scott, 1993; Krenz and Sever, 1995). To my knowledge, my study is the first to observe paedomorphic *A. talpoideum* breeding after most metamorphs have left the wetland. I speculate that these individuals did not have enough resources to mature until the end of the breeding season. While breeding earlier has been linked with higher offspring growth and survival (Ryan and Plague, 2004), the fitness benefits these late breeders gain must be greater than if they did not breed at all during that breeding season. Atlantic Coastal Plain populations lay eggs singly, while Gulf Coastal Plain populations lay eggs in clusters (Semlitsch and Walls, 1990). Although the high-elevation populations in this study are in a drainage that leads to the Gulf of Mexico, I observed females depositing eggs singly.

Coexistence of *A. talpoideum* with fish is uncommon (Petranka, 1998). Bluegill (*Lepomis macrochirus*) is a notable predator of *A. talpoideum* eggs and larvae in South Carolina (Semlitsch, 1988). At my field sites, I observed *A. talpoideum* in wetlands that also contain redbreast sunfish (*Lepomis auritus*) and creek chubs (*Semotilus atromaculatus*). This coexistence may be possible in the high-elevation *A. talpoideum* populations because the habitat in these wetlands contains abundant refuges (leaf packs and aquatic vegetation), which could make it complex enough to stabilize predator-prey interactions (Huffaker, 1958). Presence of predatory fish has been shown to reduce *A. talpoideum* activity in the water column and decrease growth (Semlitsch, 1987b), and it has also been shown to decrease the number of larvae that mature as paedomorphs (Jackson and Semlitsch, 1993). The small mouth size of these fish at these sites may also explain this coexistence. I speculate that these fish are likely eating eggs and small larvae or paedomorphs, but their mouths may be too small to swallow large paedomorphs. If

this is true, the presence of fish in these wetlands likely reduces the growth and survival of small individuals, while probably having no direct effect on survival of larger individuals.

In the mesocosm experiment, temperature did not affect body size prior to metamorphosis. Therefore, it is not surprising that it also did not affect morphotype expression. The reason for the absence of an effect of temperature on body size is unclear. Sprules (1974) observed an increase in growth and metamorphic rates in *Ambystoma gracile* raised at 19 ºC compared to those raised at 12 ºC at constant temperatures in the laboratory. The average temperatures of the mesocosm tanks in my study (20.8 ºC and 22.7 ºC) were higher than either of these treatments, and the difference between the two treatment levels in my study was much smaller (~2 ºC). Also, the location of the mesocosm tanks in full sunlight and at a lower elevation (645 m) than the wetlands (945 – 1035 m) could have resulted in higher temperatures than natural populations experience. Larval density was not intentionally manipulated in this experiment, but unequal mortality among tanks in the first two months led to variable densities. *Ambystoma talpoideum* larval growth is highly density dependent (Semlitsch, 1987c); therefore, it was not surprising that individuals in the lowest density were larger than those in any of the higher densities.

Metamorphosis in coastal populations usually occurs in the cohort's first spring and also in the fall if the pond continues to hold water, but none of the salamanders in my experiment metamorphosed until the following spring. A similar study near the northern extent of the range of *A. talpoideum* in Kentucky observed metamorphosis in the fall and also during the cohort's second spring (Doyle and Whiteman, 2008). I suspect that salamanders in my experiment were too small to metamorphose during their first year. This could be because the climate in western North Carolina is cooler than in Kentucky and South Carolina, or because the eggs used for this experiment were collected late in the breeding season. These two scenarios may not be independent, as late breeding might be an indirect effect of the climate. Individuals that metamorphosed in my experiment had a larger average body size than those that remained paedomorphic or larval. When comparing paedomorphs to metamorphs, individuals with a larger body size prior to the spring metamorphosis event had a higher probability of metamorphosing. I interpret this as evidence of the BBL mechanism in these populations.

This study provides a combination of experimental and observational evidence supporting BBL as the selective mechanism maintaining facultative paedomorphosis in high-elevation populations of *A. talpoideum*. The environmental conditions in the aquatic environment are indicative of an environment that limits growth: low temperatures; short growing season; and presence of fish predators. Most studies on this species have been conducted in locations where the aquatic environment is favorable to growth, and have found evidence supporting the PA mechanism (Semlitsch, 1987a,b; Jackson and Semlitsch, 1993; Ryan and Semlitsch, 1998). By examining this species at high elevations, this study lends support to Whiteman's (1994) hypothesis that environmental differences among populations of the same species can result in different mechanisms for the selection and maintenance of facultative paedomorphosis.

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CHAPTER II: COULD AGGRESSION BY A TERRESTRIAL SALAMANDER DETER METAMORPHOSIS IN A FACULTATIVELY PAEDOMORPHIC SALAMANDER?

INTRODUCTION

Competition between species is ubiquitous in the natural world and can influence aspects of an organism's physiology, ecology, and evolutionary trajectory (Connell, 1961; 1983; Pfenning et al., 2007; Rabosky, 2013). In animals, competition is usually mediated by aggressive behavior (Pieman and Robinson, 2010). Conspecific aggression can affect fitness through various mechanisms (Elliot, 1986; Lahti et al., 2001), but heterospecific aggression usually limits fitness through exclusion from resources. Competitive exclusion via aggression consists of one species interfering with another species' ability to exploit a common resource (Crombie, 1947). Aggressive exclusion from food or space resources has been observed in fish (Sabo and Pauley, 1997), birds (Rome and Ellis, 2004), mammals (Tannerfeldt et al., 2002), and amphibians (Hairston, 1980).

Polyphenisms are discrete morphotypes that are a product of organisms' responses to environmental cues. They are excellent models for examining how the environment influences the evolution of phenotypic plasticity (West-Eberhard, 1989). Facultative paedomorphosis is a polyphenism exhibited in some salamander species, in which aquatic larvae either metamorphose into a terrestrial subadult or mature in the larval aquatic body form as a paedomorph. The morphotype that a larva embodies is a prediction of which body form, along with its requisite habitat, will maximize fitness. Therefore, the relative quality of the terrestrial and aquatic habitats affects the pathway that an individual takes (Whiteman, 1994).

The mole salamander (*Ambystoma talpoideum*) is a well-studied facultatively paedomorphic salamander in the southeastern United States (Petranka, 1998). Inter- and intraspecific competition of *A. talpoideum* during the larval stage has been studied (Semlitsch and Walls, 1993; Walls, 1996); as have the effects of larval competition on the production of the alternative morphotypes (Anderson and Whiteman,

2015). However, I am aware of no study that has explored the possibility that interspecific competition in the terrestrial environment may affect the fitness tradeoffs involved in facultative paedomorphosis. This could be because most locations where *A. talpoideum* has been studied (e.g., the South Carolina Coastal Plain) do not have extensive salamander communities in the terrestrial habitat. The southern Appalachian Mountains have a great diversity of salamanders, especially fully terrestrial species in the genus *Plethodon* (Petranka, 1998). In the Nantahala Mountains of western North Carolina, a disjunct *A. talpoideum* population is sympatric with a high-elevation endemic *Plethodon.* This species, *Plethodon shermani,* exhibits highly aggressive behaviors towards conspecifics and a closely related species (Drummond, 2015). *Plethodon* densities in this region are high and can range from 1.57 – 7.32 salamanders/m^2 (Pursel, 2012). If this highly abundant terrestrial species competes with *A. talpoideum* for burrow space, aggression between these two species is likely. Competitive exclusion via aggression could reduce the ability of metamorphic *A. talpoideum* to acquire resources and survive, which would affect their fitness. This interaction could affect the relative fitness payoffs of metamorphosis and paedomorphosis and be an important driver of the propensity to metamorphose. This system offers a unique opportunity to explore the potential effects of competition in the terrestrial habitat on the maintenance of facultative paedomorphosis.

MATERIALS AND METHODS

Aggression trials between metamorphic *A. talpoideum* and *P. shermani* were conducted to explore the potential for competition between these species. Study subjects were collected within 75 m of Whiteoak Bottoms wetland in the Nantahala Mountains of Macon County, North Carolina. I collected animals during *A. talpoideum* breeding migrations to the wetland (October – December 2014) and away from the wetland (March – June 2015) when both species were readily available. *Plethodon shermani* hybridizes along an elevational gradient with *P. teyahalee*, and *P. shermani* occurs at the upper range of this hybrid zone. Hybrids were infrequently observed at this study site, and only individuals with color phenotypes indicative of genetically "pure" *P. shermani* (color score: red ≥ 2 and white = 0; Hairston et al., 1992) were used in this experiment.

Animals were housed at Western Carolina University in Cullowhee, North Carolina in separate plastic containers (29 x 16 x 9 cm) lined with moistened, unbleached paper towels and moss. Salamanders were exposed to a photoperiod matching actual day length (10 – 12 hours) and each fed two invertebrates at the beginning of a five-day period, which allowed each salamander to establish a territory (Jaeger, 1981). The paper towel and moss were sprayed with well water every other day as needed to maintain moisture.

From 5 November 2014 to 21 June 2015 aggression trials were conducted under dim red light during the period of peak terrestrial salamander activity (1 – 3 hours into the dark cycle; Maerz et al., 2001). Residents of both species were either paired with an intruder of the same species (conspecific trials) or the other species (heterospecific trials). Trials were initiated when the moss was removed, and an intruder was randomly selected and placed on the opposite side of an opaque barrier from the resident. The resident was then picked up and placed back into the container to control for any effect of handling on the behavior of the salamanders. After a five-minute acclimation period, the opaque barrier was removed, and the behaviors of the salamanders were observed for 30 minutes. Aggressive behaviors observed were bite (opening and closing the jaws on the opponent) and snap (opening and closing the

jaws without contacting the opponent; Nishikawa, 1985). I only tabulated behaviors that are clearly classifiable as aggression. Signaling behaviors, such as look towards, used in previous studies on intraspecific aggression (Nishikawa, 1985) may not be interpretable by divergent species and may not be reliable indicators of inter-genera aggression. I examined the behavior of the residents, because residents show higher levels of aggression than intruders (Krebs, 1982; Mathis et al., 1998). I used Fisher's exact test to examine differences in the presence or absence of aggression between conspecific and heterospecific trials. Salamanders were used in only one trial to avoid effects from previous encounters.

RESULTS

Resident *P. shermani* were paired with *A. talpoideum* intruders (n = 15) and conspecific intruders (n = 6). Resident *A. talpoideum* were paired with *P. shermani* intruders (n = 20) and conspecific intruders (n = 6). In *P. shermani* resident trials, 6.7% of heterospecific trials resulted in resident aggression, while 33.3% of conspecific trials resulted in resident aggression (Table 2). Fisher's exact test for *P. shermani* resident trials was not statistically significant (P = 0.18). In *A. talpoideum* resident trials, resident aggression was observed in 10% of heterospecific trials and 16.7% of conspecific trials (P = 1.0; Table 3).

Table 2. Number and percentage of *Plethodon shermani* resident trials resulting in aggression (bite or snap) or no aggression by resident.

	Intruder species	
Outcome	*Plethodon shermani*	*Ambystoma talpoideum*
Aggression	2 (33.3%)	1 (6.7%)
No aggression	4 (66.7%)	14 (93.3%)
Total (n)	6	15

Table 3. Number and percentage of *Ambystoma talpoideum* resident trials resulting in aggression (bite or snap) or no aggression by resident.

	Intruder species	
Outcome	*Ambystoma talpoideum*	*Plethodon shermani*
Aggression	1 (16.7%)	2 (10.0%)
No aggression	5 (83.3%)	18 (90.0%)
Total (n)	6	20

DISCUSSION

Intraspecific *A. talpoideum* trials resulted in low incidence of aggression by residents (16.7%), which is similar to findings in a previous study on this species where residents bit intruders in 14% of trials (Ducey, 1989). Intraspecific *P. shermani* trials resulted in the highest proportion of aggression by residents observed in this study. However, the observed proportion of 33% of residents biting or snapping was lower than the reported proportion in a previous study on this species (Drummond, 2015). The sample size in this study for intraspecific trials ($n = 6$ for each species) was low and could contribute to this difference. Interspecific trials with either species as the resident resulted in proportions of aggression that were lower than intraspecific trials of both species. Low levels of aggression between these species suggest that terrestrial adult *A. talpoideum* and *P. shermani* probably do not compete for resources.

I was not able to test interactions between *P. shermani* and juvenile *A. talpoideum* metamorphs, because I only encountered adults. It is possible that *P. shermani* react differently towards juvenile *A. talpoideum*, but this study cannot address that question. The low levels of aggression between *A. talpoideum* and *P. shermani* may be due to spatial partitioning of burrow habitat. *Ambystoma talpoideum* uses shallow burrows in the upper 10 cm of the soil (Semlitsch, 1981), but *P. shermani* likely uses much deeper burrows. A closely related species, *P. jordani*, has been reported to use burrows at least 45 cm deep (Brooks, 1946). Spatially-varying environments and spatial niche partitioning promote coexistence of species (Chesson, 2000a,b). Therefore, differential use of burrow space may prevent competition and aggression between these two species.

Few studies have assessed competition between the genera *Ambystoma* and *Plethodon*. One study between the relatively large species, *A. maculatum*, and the relatively small species, *P. cinereus*, observed numerous bites by *A. maculatum*, that occasionally resulted in predation (Ducey et al., 1994). The authors could not determine if attacks by *A. maculatum* were aggression or attempted predation, but they noted that posture of *A. maculatum* during attacks on *P. cinereus* was similar to *A. maculatum* posture when feeding on invertebrate prey. The two species used in my experiment are much more closely matched in

size, so predation is not a possibility between adults. Generalizing the lack of aggression between *A. talpoideum* and *P. shermani* observed in my study may suggest that competition between other species in these two genera is unlikely. This lends credence to the idea that interaction between *A. maculatum* and *P. cinereus* is better classified as predation.

This study did not find any evidence for the potential of interspecific interference competition causing a reduction in the fitness of terrestrial *A. talpoideum* relative to aquatic adults. I speculate that the divergent species in this study may not overlap in microhabitat use. Future studies could consider how the fitness tradeoffs in facultative paedomorphosis are affected by terrestrial competition with a closely related species, such as *Ambystoma maculatum*.

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APPENDIX

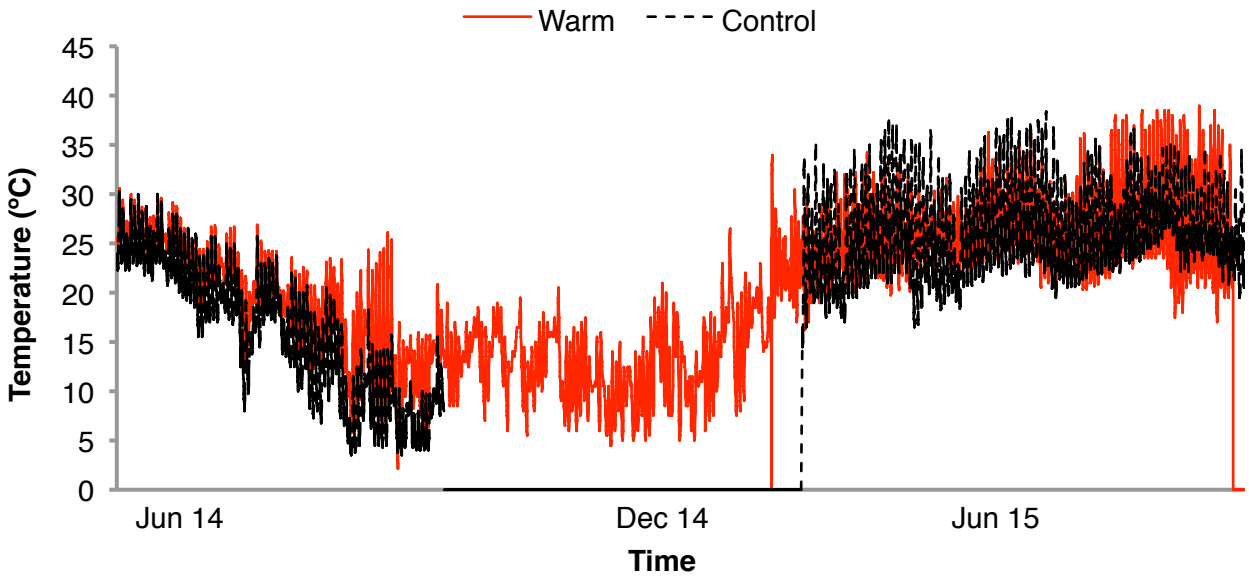

Fig. A1. Average temperature data for the two treatment levels throughout the experiment for available dates. Some control data is missing during winter 2014 – 2015 due to data rollover on data loggers.

Table A1. Complete data from standardized dipnet surveys in both wetlands.

Date	Wetland	SVL (mm)	Total length (mm)	Mass (g)	Sex	Notes
5/27/14	Whiteoak Bottoms	38	69	ND	j	maybe female
6/3/14	Whiteoak Bottoms	46	85	3.87	ND	
6/3/14	Whiteoak Bottoms	49	89	4.7	ND	
6/3/14	Whiteoak Bottoms	53	91	5.6	ND	
6/6/14	Thunderstruck	48	95	5.74	ND	
6/6/14	Thunderstruck	46	93	4.49	ND	
6/6/14	Thunderstruck	45	92	4.1	ND	
6/6/14	Thunderstruck	44	85	3.59	ND	
6/6/14	Thunderstruck	48	91	4.48	ND	
6/6/14	Thunderstruck	47	93	5.06	ND	
6/6/14	Thunderstruck	49	93	4.82	ND	
6/6/14	Thunderstruck	45	91	4.2	ND	
6/6/14	Thunderstruck	47	96	4.63	ND	
6/6/14	Thunderstruck	48	91	4.59	ND	

6/6/14	Thunderstruck	43	83	3.52	ND	
7/30/14	Whiteoak Bottoms	52	69	6.66	f	partial tail
7/30/14	Whiteoak Bottoms	23	45	0.8	j	
7/30/14	Whiteoak Bottoms	23	41	0.86	j	
7/30/14	Whiteoak Bottoms	23	37	0.72	j	
7/30/14	Whiteoak Bottoms	22	44	0.58	j	
7/30/14	Whiteoak Bottoms	24	43	0.61	j	
8/14/14	Thunderstruck	46	89	5.1	ND	
8/14/14	Thunderstruck	49	94	5.76	ND	
8/14/14	Thunderstruck	47	90	5.41	f	
8/14/14	Thunderstruck	51	96	6.39	ND	
8/14/14	Thunderstruck	47	98	6.26	m	
8/14/14	Thunderstruck	48	96	5.41	f	
8/14/14	Thunderstruck	24	50	0.84	j	
8/14/14	Thunderstruck	24	55	0.88	j	
8/14/14	Thunderstruck	22	42	0.49	j	
8/14/14	Thunderstruck	24	48	0.79	j	
8/14/14	Thunderstruck	25	49	0.85	j	
8/14/14	Thunderstruck	25	53	0.89	j	
8/14/14	Thunderstruck	25	54	0.88	j	
8/14/14	Thunderstruck	25	50	0.78	j	
8/14/14	Thunderstruck	25	43	0.59	j	
8/14/14	Thunderstruck	19	39	0.53	j	
8/14/14	Thunderstruck	20	41	0.74	j	
8/14/14	Thunderstruck	18	29	0.31	j	
8/14/14	Thunderstruck	20	36	0.38	j	
9/6/14	Whiteoak Bottoms	33	64	2.03	j	
9/6/14	Whiteoak Bottoms	31	59	2.03	j	
9/6/14	Whiteoak Bottoms	54	54	8.52	f	
10/15/14	Whiteoak Bottoms	37	52	1.61	j	no tail tip
10/15/14	Whiteoak Bottoms	39	74	2.35	j	
10/15/14	Whiteoak Bottoms	55	110	6.93	f	
10/15/14	Whiteoak Bottoms	67	116	9.49	f	
11/19/14	Thunderstruck	34.4	69.7	2.38	j	
11/19/14	Thunderstruck	47.1	84.9	4.29	f	
11/19/14	Thunderstruck	ND	ND	ND	m	metamorph
12/11/14	Whiteoak Bottoms	53	96	5.76	f	
12/11/14	Whiteoak Bottoms	39	67	2.35	j	
12/11/14	Whiteoak Bottoms	41	75	2.61	j	
12/11/14	Whiteoak Bottoms	37	69	2	j	
12/11/14	Whiteoak Bottoms	44	82	3.21	j	
12/11/14	Whiteoak Bottoms	43	81	2.82	j	
12/11/14	Whiteoak Bottoms	46	85	2.89	j	jaw deformed
1/20/15	Thunderstruck	40	75	2.38	j	
1/20/15	Thunderstruck	57	96	5.27	f	
3/1/15	Thunderstruck	39	72	2.35	f	
3/1/15	Whiteoak Bottoms	NA	NA	NA	NA	none found
4/6/15	Whiteoak Bottoms	NA	NA	NA	NA	none found
4/22/15	Thunderstruck	52	97	4.08	f	
4/22/15	Thunderstruck	45	88	3.9	f	

4/22/15	Thunderstruck	53	94	5.94	f
4/22/15	Thunderstruck	49	92	4.65	f
4/22/15	Thunderstruck	57	111	8.7	f
4/22/15	Thunderstruck	55	101	6.73	m
4/22/15	Thunderstruck	56	103	7.17	f
4/22/15	Thunderstruck	57	103	7.35	f
4/22/15	Thunderstruck	57	102	7.73	f
4/22/15	Thunderstruck	41	81	2.81	f
4/22/15	Thunderstruck	55	100	6.34	f
4/22/15	Thunderstruck	61	108	8.22	f
4/22/15	Thunderstruck	53	101	5.27	m
4/22/15	Thunderstruck	47	98	3.91	f
4/22/15	Thunderstruck	55	103	6.06	f
4/22/15	Thunderstruck	58	103	7.9	m
4/22/15	Thunderstruck	55	105	7.33	f
4/22/15	Thunderstruck	56	106	6.58	f
4/22/15	Thunderstruck	60	110	7.02	m
4/22/15	Thunderstruck	50	97	4.89	f
4/22/15	Thunderstruck	57	106	6.55	m
4/22/15	Thunderstruck	57	101	5.56	m

ND = no data; NA = not applicable; j = juvenile (larva); f = female; m = male